Docket No. 218472US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Bettina MOECKEL, et al.

SERIAL NO: 10/075,460 GAU: 1645

FILED: February 15, 2002 EXAMINER:

FOR: NUCLEOTIDE SEQUENCES WHICH CODE FOR THE rpsl GENE

INFORMATION DISCLOSURE STATEMENT UNDER 37 CFR 1.97

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Applicant(s) wish to disclose the following information.

REFERENCES

☐ The applicant(s) wish to make of record the references listed on the attached form PTO-1449. Copies of the listed references are attached, where required, as are either statements of relevancy or any readily available English translations of pertinent portions of any non-English language references.

☐ A check is attached in the amount required under 37 CFR §1.17(p).

RELATED CASES

☒ Attached is a list of applicants' pending application which may be related to the present application. A copy of the claims and drawings of the pending application is attached .

☐ A check is attached in the amount required under 37 CFR §1.17(p).

CERTIFICATION

☐ Each item of information contained in this information disclosure statement was first cited in a communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this statement.

☐ No item of information contained in this information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application or, to the knowledge of the undersigned, having made reasonable inquiry, was known to any individual designated in 37 CFR §1.56(c) more than three months prior to the filing of this statement.

DEPOSIT ACCOUNT

☒ Please charge any additional fees for the papers being filed herewith and for which no check is enclosed herewith, or credit any overpayment to deposit account number 15-0030. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

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LIST OF RELATED CASES

Docket Number	Serial or Patent Number	Filing or Issue Date	Inventor/ Applicant
215482US0X	10/058,945	01/30/02	HERMANN, et al.
218472US0X*	10/075,460	02/15/02	MOECKEL, et al.

*Present Application; listed for information
NFO/sbr

WHAT IS CLAIMED IS:

1. An isolated polynucleotide, which encodes a protein comprising the amino acid sequence of SEQ ID NO:2.

2. The isolated polynucleotide of Claim 1, wherein said protein has trehalose 6-phosphate synthase activity.

3. A vector comprising the isolated polynucleotide of Claim 1.

4. A host cell comprising the isolated polynucleotide of Claim 1.

5. The host cell of Claim 4, which is a *Coryneform* bacterium.

6. The host cell of Claim 4, wherein said host cell is selected from the group consisting of *Coryneform glutamicum, Corynebacterium acetoglutamicum, Corynebacterium acetoacidophilum, Corynebacterium melassecola, Corynebacterium thermoaminogenes, Brevibacterium flavum, Brevibacterium lactofermentum,* and *Brevibacterium divaricatum*.

7. A method for detecting a nucleic acid with at least 70% homology to nucleotide of Claim 1, comprising contacting a nucleic acid sample with a probe or primer comprising at least 15 consecutive nucleotides of the nucleotide

sequence of Claim 1, or at least 15 consecutive nucleotides of the complement thereof.

8. A method for producing a nucleic acid with at least 70% homology to nucleotide of Claim 1, comprising contacting a nucleic acid sample with a primer comprising at least 15 consecutive nucleotides of the nucleotide sequence of Claim 1, or at least 15 consecutive nucleotides of the complement thereof.

9. A process for screening for polynucleotides, which encode a protein having trehalose 6-phosphate synthase activity comprising

a) hybridizing the isolated polynucleotide of Claim 1 to the polynucleotide to be screened;

b) expressing the polynucleotide to produce a protein; and

c) detecting the presence or absence of trehalose 6-phosphate synthase activity in said protein.

10. A method for making a trehalose 6-phosphate synthase protein, comprising culturing the host cell of Claim 4 for a time and under conditions suitable for expression of the trehalose 6-phosphate synthase protein; and collecting the trehalose 6-phosphate synthase protein.

11. An isolated polynucleotide, which comprises SEQ ID NO:1.

12. An isolated polynucleotide, which is complimentary to the polynucleotide of Claim 11.

13. An isolated polynucleotide, which is at least 70% identical to the polynucleotide of Claim 11.

14. An isolated polynucleotide, which is at least 80% identical to the polynucleotide of Claim 11.

15. An isolated polynucleotide, which is at least 90% identical to the polynucleotide of Claim 11.

16. An isolated polynucleotide, which comprises at least 15 consecutive nucleotides of the polynucleotide of Claim 11.

17. An isolated polynucleotide, which hybridizes under stringent conditions to the complementary polynucleotide of Claim 11; wherein said stringent conditions comprise washing in 5X SSC at a temperature from 50 to 68°C.

18. The isolated polynucleotide of Claim 11, which encodes a protein having trehalose 6-phosphate activity.

19. A vector comprising the isolated polynucleotide of Claim 11.

20. A host cell comprising the isolated polynucleotide of Claim 11.

21. The host cell of Claim 20, which is a *Coryneform* bacterium.

22. The host cell of Claim 20, wherein said host cell is selected from the group consisting of *Coryneform glutamicum, Corynebacterium acetoglutamicum, Corynebacterium acetoacidophilum, Corynebacterium melassecola, Corynebacterium thermoaminogenes, Brevibacterium flavum, Brevibacterium lactofermentum,* and *Brevibacterium divaricatum*.

23. A process for screening for polynucleotides, which encode a protein having trehalose 6-phosphate synthase activity comprising

a) hybridizing the isolated polynucleotide of Claim 11 to the polynucleotide to be screened;

b) expressing the polynucleotide to produce a protein; and

c) detecting the presence or absence of trehalose 6-phosphate synthase activity in said protein.

24. A process for screening for polynucleotides, which encode a protein having trehalose 6-phosphate synthase activity comprising

a) hybridizing the isolated polynucleotide of Claim 11 to the polynucleotide to be screened;

b) expressing the polynucleotide to produce a protein; and

c) detecting the presence or absence of trehalose 6-phosphate synthase activity in said protein

25. A method for detecting a nucleic acid with at least 70% homology to nucleotide of Claim 11, comprising contacting a nucleic acid sample with a probe or primer comprising at least 15 consecutive nucleotides of the nucleotide sequence of Claim 11, or at least 15 consecutive nucleotides of the complement thereof.

26. A method for producing a nucleic acid with at least 70% homology to nucleotide of Claim 11, comprising contacting a nucleic acid sample with a primer comprising at least 15 consecutive nucleotides of the nucleotide sequence of Claim 11, or at least 15 consecutive nucleotides of the complement thereof.

27. A method for making a trehalose 6-phosphate synthase protein, comprising

a) culturing the host cell of Claim 20 for a time and under conditions suitable for expression of the trehalose 6-phosphate synthase protein; and

b) collecting the trehalose 6-phosphate synthase protein.

28. A *Coryneform* bacterium, which comprises an attenuated *otsA* gene.

29. The *Coryneform* bacterium of Claim 28, wherein said *otsA* gene comprises the nucleotide sequence of SEQ ID NO:1.

30. The *Coryneform* bacterium of Claim 28, wherein said *otsA* gene comprises a nucleotide sequence that hybridizes under stringent conditions to a polynucleotide that is complimentary to SEQ ID NO:1, wherein said stringent conditions comprise washing in 5X SSC at a temperature of from 50 to 68°C.

31. *Corynebacterium glutamicum* DSM 14041.

32. A process for producing L-amino acids comprising culturing a bacterial cell in a medium suitable for

producing L-amino acids, wherein said bacterial cell comprises an attenuated *otsA* gene.

33. The process of Claim 32, wherein said bacterial cell is a *Coryneform* bacterium or *Brevibacterium*.

34. The process of Claim 33, wherein said bacterial cell is selected from the group consisting of *Coryneform glutamicum, Corynebacterium acetoglutamicum, Corynebacterium acetoacidophilum, Corynebacterium melassecola, Corynebacterium thermoaminogenes, Brevibacterium flavum, Brevibacterium lactofermentum,* and *Brevibacterium divaricatum*.

35. The process of Claim 32, wherein said *otsA* gene comprises the nucleotide sequence of SEQ ID NO:1.

36. The process of Claim 32, wherein said *otsA* gene comprises a nucleotide sequence that hybridizes under stringent conditions to a polynucleotide that is complimentary to SEQ ID NO:1, wherein said stringent conditions comprise washing in 5X SSC at a temperature of from 50 to 68°C.

37. The process of Claim 32, wherein said L-amino acid is L-lysine.

38. The process of Claim 32, wherein said bacteria further comprises at least one gene whose expression is enhanced, wherein said gene is selected from the group consisting of *dapA*, *gap*, *eno*, *tpl*, *pgk*, *zwf*, *pyc*, *mqo*, *lysC*, *lysE*, and *zwa 1*.

39. The process of Claim 32, wherein said bacteria further comprises at least one gene whose expression is attenuated, wherein said gene is selected from the group consisting of *pck*, *pgi*, *poxB*, *zwa2*, *fda*, *hom*, *thrB*, and *panD*.

40. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2.

41. An isolated polypeptide, which has an amino acid sequence that is at least 90% identical to SEQ ID NO:2.

42. An isolated polynucleotide consisting essentially of SEQ ID NO:1.

43. A vector comprising the isolated polynucleotide of Claim 42.

44. A host cell comprising the isolated polynucleotide of Claim 42.

45. A method of making a trehalose 6-phosphate synthase protein, comprising culturing the host cell of Claim 44

for a time and under conditions suitable for expression of the trehalose 6-phosphate synthase protein; and collecting said trehalose 6-phosphate synthase protein.

ABSTRACT OF THE DISCLOSURE

The present invention provides nucleotide sequences from *Coryneform* bacteria which code for the OtsA protein and processes for the fermentative preparation of amino acids using bacteria in which the otrA gene is attenuated.

Figure 1: Plasmid pUC18otsA

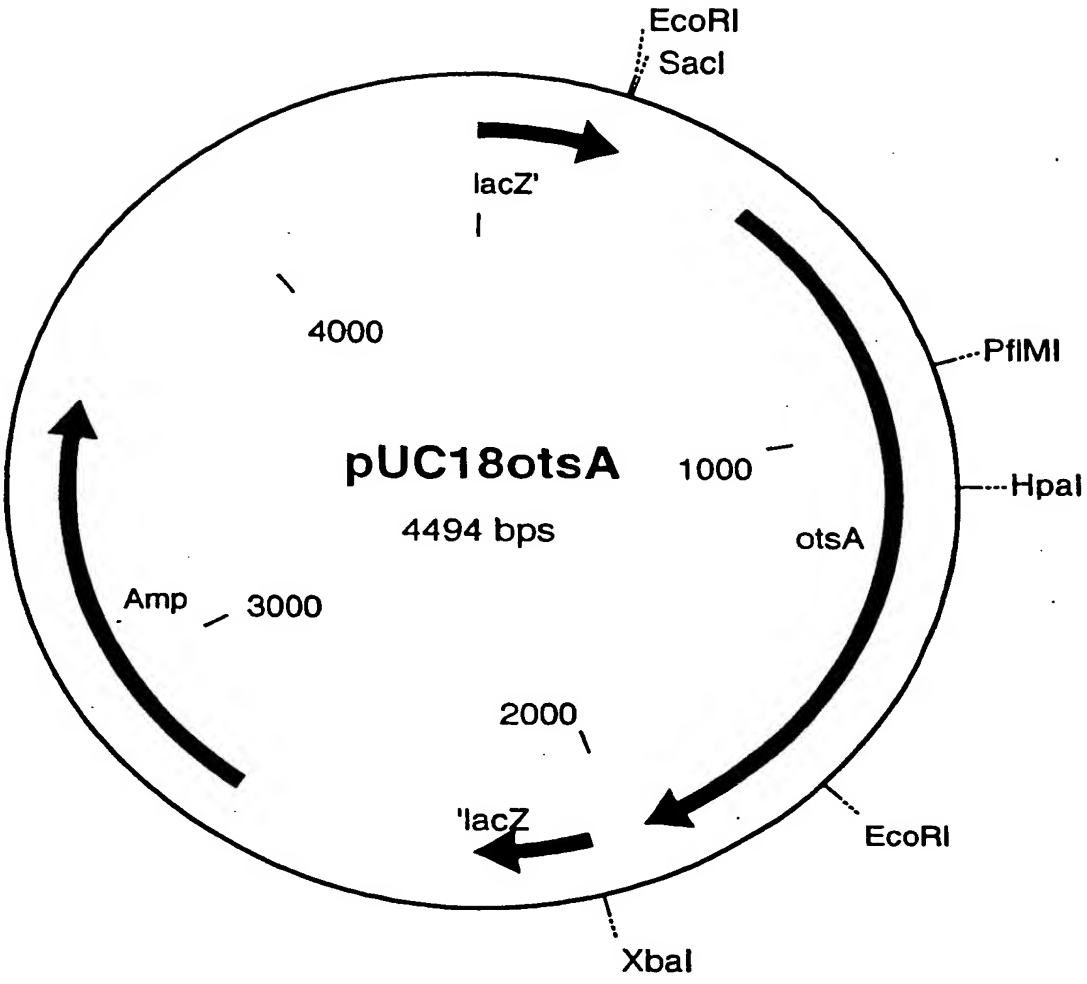

Figure 2: Plasmid pK19mobsacBΔotsA